bio-protocol Preprint

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Islet dispersion

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Updated date: Mar 2, 2022

An abbreviated version of this protocol was published in Science Advances in Mar 2021

GIP mediates the incretin effect and glucose tolerance by dual actions on α cells and β cells

DOI: 10.1126/sciadv.abf1948

Detailed protocol

Supplies
Islet culture media - RPMI w 10% FBS (4C)
DNAse (2,727, -20C aliquots, current aliquot @ 4C)
HEPES (1M, 4C)
Accutase (4C, aliquoted stocks at -20C)
Filters: Celltrics 30 um (green)

General protocol

1. Isolate islets as normal, allow to recover overnight, collect islets to 1.5 ml epi tube—note the number—with 0.5-1 mL PBS
2. spin down islets at 300xg in PBS
3. discard the supernatant, add 300 ul accutase and incubate at 37 C waterbath for 15 minutes, vortex/pipet 3-4x during incubation
4. pipet up and down and make sure there is no visible cell clumps.
5. add 3X volume (900 ul) of islet culture medium, spin down for 3.5 minutes at 350g @ 4C
6. remove the supernatant, gently tab the tube and add 500ul sorting buffer, pipet up and down
7. spin down at 350g 4C for 3.5 minutes, wash again with 300-500 ul sorting buffer
8. Add 300 ul sorting buffer, filter through 30 micron tube into falcon tube for sorting. Rinse tube with 300 ul sorting buffer
9. keep on ice and protected from light, take to FACS sorter asap

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Campbell, J. (2022). Islet dispersion. Bio-protocol Preprint. bio-protocol.org/prep1552.
2. El, K., Gray, S. M., Capozzi, M. E., Knuth, E. R., Jin, E., Svendsen, B., Clifford, A., Brown, J. L., Encisco, S. E., Chazotte, B. M., Sloop, K. W., Nunez, D. J., Merrins, M. J., D'Alessio, D. A. and Campbell, J. E.(2021). GIP mediates the incretin effect and glucose tolerance by dual actions on α cells and β cells. Science Advances 7(11). DOI: 10.1126/sciadv.abf1948

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